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(54) Title: CYCLIC ORGANOSELENIUM COMPOUNDS, THEIR PREPARATION AND THEIR USES			
(57) Abstract			
<p>The objects of the present invention are: novel cyclic organoseelenium compounds; the use of said novel compounds as antioxidant; pharmaceutical compositions containing them; and a method of preparation of said novel compounds. Said novel cyclic organoseelenium compounds have general formula (I).</p>			

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Cyclic organoselenium compounds, their preparation and their uses.

5 The principal objects of the present invention are :

- novel cyclic organoselenium compounds ;
- the use of said novel compounds as antioxidant ;
- pharmaceutical compositions containing them ;
- a method of preparation of said novel compounds.

10 STATE OF THE PRIOR ART:

In aerobic organisms, during the metabolism of oxygen, very reactive entities are generated whose accumulation causes deleterious effects. These organisms possess a system of regulation, composed of enzymes and small molecules which enable controlling the production of these reactive oxygen entities. Amongst the various components of this regulation system, often called 15 Antioxidant Defence System, glutathione peroxidases play a central role in the prevention of «oxidative stress» and its deleterious consequences. These antioxidant and cytoprotecting enzymes enable degrading the endogenous or 20 exogenous cytotoxic hydroperoxides.

These enzymes catalyse the reduction of hydrogen peroxide (reaction 1) or that of organic hydroperoxides (reaction 2) by reduced glutathione (GSH):

reaction 1 :



reaction 2 :



30 The active sites of these enzymes all contain one essential selenium atom in the form of a selenocysteine residue incorporated in the polypeptide chain.

The selenium is incorporated from selenite salts, or selenate salts, or L-seleno-methionine salts arising from food (see C.K. Chow and J. Jeng; in Selenium in Medicine and Biology, M.D. Spallholz and H.E. Ganther Eds.; (1986); Academic Press). In situations of selenium deficiency in the diet, the 35 concentrations and activities of these glutathione peroxidases gradually decrease (see Y.X. Wang and J. Kiern.; Biological Trace Elements Res.; (1988); 15; 89 and

see R. Reiter and A. Wendel; *Biochem.Pharmacol.*; (1983); 32; 3063-3067); this leads to an acute susceptibility to the oxidative stress (see D.B.Coursin and H.P.Cihla, *Thorax*, (1996), 51, 479-483). The provision of selenium in the diet is therefore a limiting factor in the biosynthesis of glutathione peroxidases.

5 The protecting role of glutathione peroxidases, in situations wherein the production of hydroperoxides rises, has been demonstrated following experiments of direct intracellular micro-injection of the enzyme of erythrocyte origin which have enabled demonstrating its cytoprotective effect upon the viability of fibroblasts or endothelial cells exposed to an oxidative stress (see C. 10 Michiels *et al.*; *Experiment. Cell Res.*; (1988); 179; 581-589). On the other hand, it has been shown that the survival of human fibroblasts is appreciably lowered when glutathione peroxidase is inhibited (see C. Michiels *et al.*, *J.Eur.Biochem.*, 1988, 177, 435-441).

15 Furthermore, the glutathione peroxidases are themselves particularly sensitive to an over-production of hydroperoxides and are rapidly inhibited under these conditions (see H. Ochi *et al.*, *Arch.of Biochem.Biophys.*, (1992), 294, 2, 407-411).

20 A certain number of pathologies such as certain ischemic cardiomyopathies for example (see J.Chaudière, in *Biologie des lipides chez l'Homme*, L.Douste-Blazy and F.Mendy eds, Edition Médicale Internationale - Paris, (1988), 137-154 and see D. Vitoux *et al.*; *Ann.Biol.Clin.*; (1996); 54; 5; 181-187) are associated with a lowering of glutathione peroxidase activity.

25 The demonstration of the essential role of the selenium at the active centre of these ubiquitous enzymes (see J.T.Rotruck, in *Selenium in Biology and Medicine*, Spallholz, J.E. Martin, J.L. Ganther H.I. eds., Avi Publishing Co, Wesport, (1981), 10-16) as well as the importance of the selenium in the regulation of oxidative damages generated during certain pathologies (see Cadenas, E. and Sies, H., *Adv.Enz. Regul.*, (1985), 23, 217-237 and see Ursini, F.,Bindoli, A., *Chem. Phys.Lipids*, (1987), 44, 255-276) has enabled the 30 emergence of a novel class of organoselenium compounds as potential drugs.

Two types of compounds have been designed and prepared to this end.

On one side, modified macromolecules possessing a selenium atom introduced chemically such as selenosubtilisin (see Z.P.Wu and D.Hilvert, *J.Am.Chem.Soc.*, (1990), 112, 5647-5648) or even a seleno-abzyme (see G.M.Luo 35 *et al.*, *Biochem.Biophys.Res.Comm.*, 1994, 198, 3, 1240-1247). However, the use

of proteins with a therapeutic aim is difficult to envisage for the following reasons:

- * their biostability is often insufficient;
- * an efficient method for ensuring their intra-cellular targeting does 5 not exist;
- * they cannot be administered orally.

On the other side, synthetic molecules of low molecular weight have been synthetized, of which 2-phenyl-1,2-benziselenazolin-3-one (ebselen) (see H.Sies, Free Rad.Biol.Med., (1993), 14, 313-323) was the first compound 10 described as having a glutathione peroxidase activity. Homologs of said ebselen, i.e. 2H-3,4-dihydro-1,2-benzoselenazin-3-ones, have also been described by Pierre V. Jacquemin *et al.* in Tetrahedron Letters, (1992), Vol. 33, No. 27, 3863-3866. In fact, several organoselenium derivatives have been described as 15 glutathione peroxidase mimics, i. e. capable of reducing hydroperoxides in the presence of a biological thiol such as glutathione or lipoic acid (see I.A.Cotgreave *et al.*, Biochem.Pharmacol., (1992), 43, 793-802 and C.M.Andersson *et al.*, Free Rad.Biol.Med., (1994), 16, 17-28 and S.R.Wilson *et al.*, J.Am.Chem.Soc., (1989), 111, 5936-5939 and V.Galet *et al.*, J.Med.Chem., (1994), 37, 2903-2911). The 20 patent application WO-A-95/27706 describes compounds of benziselenazoline and benzisoselenazine structure having a glutathione peroxidase activity *inter alia*.

These organoselenium compounds, which are mimics of glutathione peroxidase, invariably produce catalytic intermediates of the selenol and/or diselenide type.

Amongst these, 2-phenyl-1,2-benziselenazolin-3-one (ebselen) and 25 some of its derivatives do not seem to have any major toxic effect (see A. Wendel *et al.*; Biochem. Pharmacol.; (1984); 33; 3241-3245 and S.D. Mercurio and G.F. Combs; Biochem. Pharmacol.; (1986); 35; 4505-4509). 2-Phenyl-1,2-benziselenazolin-3-one (ebselen) is however very little soluble in water, even in the presence of an excess of glutathione GSH, which limits its pharmacological 30 applications.

The biochemical and pharmacological properties of the organoselenium compounds which have been synthesised and studied have been recently reviewed (see M.J. Parnham and E. Graf; Progress in Drug Res.; (1991); 36; 9-47 and M.J.Parnham, Exp.Opin.Invest.Drugs, (1996), 5, 7, 861-570).

One of the aims of the present invention is to design organoselenium compounds having a catalytic activity of the glutathione peroxidase type in the presence of physiological concentrations of glutathione GSH.

5 These compounds must be able to penetrate the target tissues or cells, be soluble in water at active concentrations and must not efficiently reduce oxygen into toxic by-products.

10 These aims are attained by virtue of the present invention which resides on the design of cyclic organoselenium compounds whose antioxidant and cytoprotecting activities have been demonstrated by the Applicant and which are given below.

DESCRIPTION OF THE INVENTION:

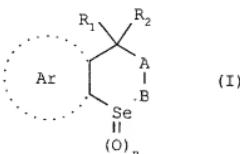
The aim of the present invention is:

15 1) to solve the novel technical problem consisting of providing novel cyclic heteroaryl selenium compounds having a very good antioxidant and cytoprotecting activity, thus constituting valuable active principles of pharmaceutical compositions;

20 2) to solve the novel technical problem above according to a solution which includes a method of preparation of these novel compounds which is easy to carry out.

25 The technical problems set forth above are solved for the first time in a simultaneous manner by the present invention in a simple way; the method of preparation of said novel compounds being relatively easy to carry out and giving good yields.

According to its first aspect, the present invention thus relates to novel cyclic organoselenium compounds, having the general formula (I) below :



in which :

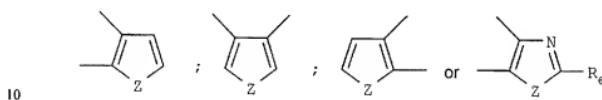
R₁ = hydrogen ; lower alkyl ; optionally substituted aryl ; optionally substituted lower aralkyl ;

R₂ = hydrogen ; lower alkyl ; optionally substituted aryl ; optionally substituted lower aralkyl ;

5 A = CO ; (CR₃R₄)_m ;

B = NR₅ ; O ; S ;

Ar = optionally substituted phenyl or an optionally substituted radical of formula :



in which :

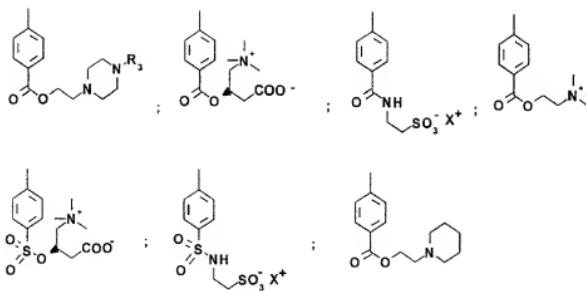
Z = O ; S ; NR₅ ;

R₃ = hydrogen ; lower alkyl ; optionally substituted aryl ; optionally substituted lower aralkyl ;

R₄ = hydrogen ; lower alkyl ; optionally substituted aryl ; optionally substituted lower aralkyl ;

R₅ = hydrogen ; lower alkyl ; optionally substituted aryl ; optionally substituted lower aralkyl ; optionally substituted heteroaryl ; optionally substituted lower heteroaralkyl ; CO(lower alkyl) ; CO(aryl) ; SO₂ (lower alkyl) ; SO₂(aryl) ;

15 R₆ = hydrogen ; lower alkyl ; optionally substituted aryl ; optionally substituted lower aralkyl ; optionally substituted heteroaryl ; optionally substituted lower heteroaralkyl ; trifluoromethyl ;



m = 0 or 1 ;

n = 0 or 1 ;

5 X⁺ represents the cation of a pharmaceutically acceptable base ;
and their pharmaceutically acceptable salts of acids or bases ;
with the proviso that :

when B = NR₅ with R₅ = hydrogen, lower alkyl, optionally substituted lower aralkyl, CO(lower alkyl), and A = CO or (-CH₂-)_m, then Ar is different from an
10 optionally substituted phenyl.

Said general formula (I) more particularly encompasses the four compounds, preparation of which is illustrated in the examples joined to this specification.

15 Said general formula (I) includes every stereoisomer, epimer and diastereoisomer, as a mixture or in isolated form.

It also includes, as indicated, the salts of pharmaceutically acceptable acids or bases of said compounds of formula (I).

Amongst the pharmaceutically acceptable acids, hydrochloric, hydrobromic, hydroiodic, sulphuric, tartaric, methanesulphonic, trifluoromethanesulphonic acid, ... can be cited in a non-limiting way.

Amongst the pharmaceutically acceptable bases, sodium hydroxide, potassium hydroxide, alkali metal or alkaline earth metal carbonates, or organic bases such as triethylamine or arginine, ... can be cited in a non-limiting way.

Within the context of the present description and annexed claims :

25 - the terms "lower alkyl and lower alkoxy (see below)" are understood as meaning straight or branched alkyl and alkoxy groups having from 1 to 8 carbon atoms ;

- the term "aryl" is understood as meaning an aromatic group selected from phenyl and naphthyl groups ;

- the term "heteroaryl" is understood as meaning a mono- or bicyclic aromatic group, each cycle, or ring, comprising five or six atoms and said cycle, or ring, or both cycles, or rings, including in its carbon skeleton from one to three heteroatoms selected from nitrogen, oxygen and sulphur ;

- the terms "lower aralkyl" and "lower heteroaralkyl" are understood as meaning, in view of the definitions above, phenyl(C₁-C₈)alkyl or naphthyl(C₁-C₈)alkyl and heteroar(C₁-C₈)alkyl respectively;

- the term "substituted" concerning the terms aryl, aralkyl, phenyl, radical (five-membered, including Z), heteroaryl, heteroaralkyl, as defined above, signifies that the groups in question are substituted on the aromatic part with one or more identical or different groups selected from the groups : (C₁-C₈)alkyl, trifluoromethyl, (C₁-C₈)alkoxy, hydroxy, nitro, amino, (C₁-C₈)alkylamino, di(C₁-C₈)alkylamino, sulphonyl, sulphonyl, sulphonamide, sulpho(C₁-C₈)alkyl, carboxyl, carbalkoxyl, carbamide (it being possible for said (C₁-C₈)alkyl groups to be linear or branched) or substituted with one or more halogen atoms.

Finally, when R₅ represents a hydrogen atom (B = -NH), the invention also includes the salts obtained with the pharmaceutically acceptable acids.

Said novel compounds have proved to be, as specified above, excellent antioxidant agents, the use of which is recommended by the Applicant in various fields. This use of said novel cyclic organoselenium compounds of the invention - compounds having formula (I) as defined above - as antioxidant agents, constitutes the second aspect of said invention.

Within the context of this second aspect, the use of said compounds of formula (I), is more particularly claimed as antioxidant agents :

- intended to be added to preserving media of grafts for transplantation of organs of human or animal origin such as the heart, the liver, the kidney and the lungs ;

and

- intended for (as active principle) the manufacture of pharmaceutical compositions with antioxidant activity, suitable especially :

* for treatments of any physiopathological condition in which an over-production of cytotoxic hydroperoxides contributes to the functional impairments of cells or tissues ; and more particularly including :

- * the treatment of inflammatory and/or ischemic cardio- and cerebro-vascular pathologies, such as the preventive and/or curative treatment of arterial restenoses following an angioplasty, the preventive and/or curative treatment of arterial stenoses following artery allografts, the treatment of intermittent 5 claudication in patients affected with obstructive ischemia of the lower members, the treatment of cerebro-vascular accidents of ischemic origin ;
 - * the treatment of inflammatory and/or ischemic digestive pathologies, such as the treatment of acute inflammations of the bowel (Crohn's disease, hemorrhagic rectocolitis) ;
- 10 * the treatment of inflammatory and/or ischemic respiratory pathologies, such as the treatment of adult respiratory distress syndrome (ARDS) and infant respiratory distress syndrome (IRDS) ;
 - * the treatment of inflammatory and/or ischemic ophthalmic pathologies, such as the treatment of glaucoma ;
- 15 * the treatment of cataracts ;
 - * the treatment of acute ophthalmic allergies ;
 - * the treatment of impairments of the retina which are associated with a macular degeneration ;
- * the treatment of viral infections causing an immuno-deficiency, such 20 as the treatment of AIDS ;
 - * the treatment of post-radiotherapy fibroses.

Generally, the potential therapeutic applications of the compounds of the invention include the treatment of any physiopathological condition in which an over-production of cytotoxic hydroperoxides contributes to the functional 25 impairments of cells or tissues. Such an over-production of hydroperoxides can be endogenous and secondary to the activation of the intra-cellular metabolic pathways such as, for example, those of the flavine or cytochrome P-450 oxygenases, those of the lipoxygenases, those of the monoamine oxidases. The over-production can also be due to the activation of the endothelial cells (xanthine 30 oxidase, 15-lipoxygenase), or of blood platelets (cyclooxygenase and 12-lipoxygenase). It can also be due to the activation, by cytokines such as TNF- α for example, of inflammatory and/or immune cells such as neutrophils, macrophages or lymphocytes for example. It may also be due to an intoxication by a free-radical generating xenobiotic. Finally, it may be due to a voluntary irradiation such as 35 practised during a radiotherapy, or an accidental irradiation.

More particularly, the second aspect of the present invention includes the use of compounds of the invention for the manufacture of pharmaceutical compositions intended for the treatment :

- * of inflammatory diseases of the bowel such as Crohn's disease or hemorrhagic rectocolitis ;
- 5 * of adult respiratory distress syndrome and infant respiratory distress syndrome ;
- * of cataracts ;
- * of AIDS ;
- 10 * of post-radiotherapy fibroses.

From the second aspect of the present invention - use of the novel compounds de formula (I) as antioxidant agents -, such as described above, comes the third aspect which is dealt with now, namely the pharmaceutical compositions containing said compounds of formula (I) as active principle.

15 Thus, according to its third aspect, the present invention relates to pharmaceutical compositions, notably having an antioxidant activity, and comprising at least one cyclic organoselenium compound of the general formula (I), or one of its pharmaceutically acceptable salts of an acid or a base, as active ingredient, optionally incorporated in a pharmaceutically acceptable excipient, carrier or vehicle.

20 Said pharmaceutical compositions of the invention, according to an advantageous embodiment, contain said active ingredient in an amount between 0.1 and 5% by weight, advantageously between 0.1 and 1 % by weight based on their total weight. According to another advantageous embodiment, said 25 compositions are in the form of unit doses comprising from 1 to 500 mg of at least one cyclic organoselenium compound of the invention (optionally incorporated in a pharmaceutically acceptable excipient, carrier or vehicle).

The pharmaceutical compositions of the invention can be formulated for, or intended for, oral, rectal or topical administration, (the compounds of 30 formula (I) may especially be formulated for ophthalmic applications in the form of an eye lotion) or even as intra-ventricular, intra-muscular, subcutaneous or intravenous injections.

The pharmaceutically acceptable excipients, vehicles and carriers which can be included in their formulation are products which are well-known to 35 the person skilled in the art and are not described in detail here.

The pharmaceutical compositions of the invention which contain the antioxidant agents disclosed by the present invention (compounds of formula (I)) are especially suitable for the treatment of any physiopathological condition in which an over-production of cytotoxic hydroperoxides contributes to the 5 functional impairments of cells or tissues ; it being possible for said over-production of hydroperoxides to be due to any one of the causes presented above in the present description, with reference to the second aspect of the invention (activation of the intra-cellular metabolic pathways, enzyme activation, macrophage or lymphocyte activation, intoxication by a free-radical generating 10 xenobiotic, voluntary or accidental irradiation).

More specifically, said pharmaceutical compositions are suitable for the treatment of the pathologies listed above in the present description (with reference to the second aspect of the invention).

It is hereby specified that the antioxidant and therapeutical or 15 pharmacological activities of the cyclic organoselenium compounds of the general formula (I) above have been demonstrated according to safe and reliable tests well-known to the person skilled in the art, which comprise :

- a/ measuring the glutathione peroxidase activity;
- b/ measuring the cytoprotective effect in human umbilical vein 20 endothelial cells.

It is hereby incidentally mentioned that the preparation of pharmaceutical compositions incorporating an effective amount of at least one organoselenium compound of formula (I) according to the invention as well as the therapeutical treatments implying the use of such a compound make up an integral 25 part of the present invention.

According to its last aspect, given below, the invention even relates to a method of preparation of said organoselenium compounds of formula (I). Said method comprises the following essential steps :

- a/ preparing or using an orthohalo(hetero)arylacetonitrile derivative, 30 optionally mono- or gem- disubstituted in the benzylic position; then, according to the series considered :
 - for the preparation of said compounds of formula (I) in which $A=(CR_3R_4)_m$ with $m = 0$ (A does not exist) and $n = 0$:
 - b1/ hydrolyzing said nitrile derivative into an amide derivative,
 - c1-1/ transforming this amide derivative into an amine derivative 35 by a transposition reaction according to conventional methods,

d1-1/ allowing said amine derivative to react with a nucleophilic selenium derivative, optionally generated *in situ*, in the presence of a copper Cu(I) salt, in a polar organic solvent, to lead to the corresponding (hetero)aryliselenazoline derivative,

5 e1-1/ optionally, N-alkylating or N-arylating or N-acylating or N-sulphonylating, according to conventional procedures, said (hetero)aryliselenazoline derivative;

- for the preparation of said compounds of formula (I) in which A = CO and n = 0 :

10 b1/ hydrolyzing said nitrile derivative into an amide derivative,

c1-2/ allowing said amide derivative to react with a nucleophilic selenium derivative, optionally generated *in situ*, in the presence of a copper Cu(I) salt, in a polar organic solvent, to lead to the corresponding (hetero)aryliselenazone derivative,

15 d1-2/ optionally, N-alkylating or N-arylating or N-acylating or N-sulphonylating, said (hetero)aryliselenazone derivative;

- for the preparation of said compounds of formula (I) in which A = CH₂ and n = 0 :

20 b2/ reducing said nitrile derivative into an amine derivative with the aid of borane for example in an ethereal solvent such as tetrahydrofuran for example,

c2/ allowing said amine derivative to react with a nucleophilic selenium derivative, optionally generated *in situ*, in the presence of a copper Cu(I) salt, in a polar organic solvent, to lead to the corresponding (hetero)aryliselenazine derivative,

25 d2/ optionally, N-alkylating or N-arylating or N-acylating or N-sulphonylating, according to conventional procedures, said (hetero)aryliselenazine derivative;

- for the preparation of said compounds of formula (I) in which A = (CR₃R₄)
(≠ CH₂) and n = 0 :

b3/ carrying out a mono- or a bis-C-alkylation of said nitrile derivative according to conventional methods, with the aid of an organolithium derivative for example, in an ethereal solvent such as tetrahydrofuran;

30 c3/ allowing the amine derivative obtained to react with a nucleophilic selenium derivative, optionally generated *in situ*, in the presence of a

copper Cu(I) salt, in a polar organic solvent, to lead to the corresponding (hetero)arylisoselenazine derivative;

5 d3/ optionally, N-alkylating or N-arylation or N-acylating or N-sulphonylating, according to conventional procedures, said corresponding (hetero)arylisoselenazine derivative;

10 finally, optionally, for the preparation of compounds of formula (I) in which n = 1, Se-oxidising, according to conventional procedures, the corresponding derivative of formula (I) (wherein n = 0) obtained previously according to any one of the synthetic routes above.

15 Said method is close to that described in the application WO-A-95/27706. In a way, it constitutes an adaptation of said method according to WO-A-95/27706 for obtaining heteroaromatic derivatives. The implementation of each of its steps does not give rise to any particular difficulty to the person skilled in the art.

20 According to the advantageous variants of implementation of said method:

15 - the nucleophilic selenium derivative (which intervenes in steps d1-1/, c1-2/, c2/ and c3/) is a selenocyanate salt, such as potassium selenocyanate for example, which can be :

25 * either generated *in situ* from selenium metal Se(0) and a cyanide salt, such as potassium cyanide for example,

 * or added to the reaction medium as such ;

20 - the copper salt Cu(I) (which intervenes in the same steps) is cuprous iodide ;

25 - the polar organic salt (which intervenes in the same steps) is dimethylformamide ;

30 - the intervening oxidant for the Se-oxidation is either peracid, such as meta-chloroperbenzoic acid or hydrogen peroxide.

 Other aims, characteristics and advantages of the invention will appear clearly in the light of the following explanatory description made with reference to various non-limiting examples given solely as illustration and which in no way limit the scope of the invention. In the examples, all percentages are given by weight unless otherwise indicated.

35 In the annexed figures,

 - Figure 1 shows the inhibition of the TNF- α -induced interleukin 8 release by endothelial cells (HUVEC);

- Figure 2 shows the inhibition of the TNF- α -induced P-selectin expression by endothelial cells (HUVEC);

- Figure 3 shows the inhibition of the TNF- α -induced E-selectin expression by endothelial cells (HUVEC).

5

EXPERIMENTAL SECTION:

I Synthesis

10 All reactions were carried out under an inert nitrogen atmosphere unless otherwise indicated.

Mass spectra were recorded on a Nermag R10-10B instrument. Ionisation used is either electron impact (EI) at 70 electron-volts or chemical ionisation (CI) in ammonia or isobutene, or fast atom bombardment (FAB) on a glycerol matrix.

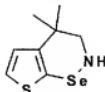
15 The ^1H and ^{13}C NMR spectra were recorded on a Varian Gemini-200 instrument, the ^{77}Se NMR spectra on a Bruker AMX 500 instrument. The chemical shifts are given in ppm with respect to tetramethylsilane (^1H and ^{13}C NMR spectra) or to dimethylselenide (^{77}Se NMR spectra). The multiplicities are expressed as follows: "s" for singlet, "bs" for broad singlet, "d" for doublet, "t" for triplet, "q" for quadruplet and "m" for multiplet; "E" for even and "O" for odd.

20 The melting points (m.p. °C) were recorded on a Gallenkamp instrument and are given uncorrected.

Purification by liquid column chromatography was carried out with Merck® Si60 F₂₅₄.

25

EXAMPLE 1: Preparation of 4,4-dimethyl-thieno-[3,2-e]-isoselenazine :



30 BXT 51097

A/ Preparation of 2-bromo-3-bromomethyl-thiophene :

This compound is prepared from 2-bromo-3-methyl-thiophene (1.85 g; 10.4 mmoles) according to a method identical to that described in the literature by

E. Campaigne and W.M. Lesuer (J.Am.Chem.Soc., 1949, 71, 333-335), and is obtained as a colourless oil (2.45 g). The product is used as such in the next step.

Yield: 95 %

Physical characteristics :

* NMR ^1H : (CDCl_3)

4.43 ppm (s, 2H); 6.98 ppm (d, 1H, $J=5.7$ Hz); 7.24 ppm (d, 1H, $J=5.7$ Hz).

B/ Preparation of 3-(2-bromo)-thienyl-acetonitrile:

A round-bottomed flask containing the preceding bromo derivative.

(2.10 g ; 8.2 mmoles) dissolved in methanol (8 ml), is cooled in a water bath. Under an inert atmosphere, sodium cyanide (0.610 g ; 12.3 mmoles) is added all at once. The mixture is left for 4 hours at ambient temperature. The solvent is evaporated under reduced pressure. The residue is taken up into 15 ml of water and extracted with 2 x 15 ml of dichloromethane. The organic phases are combined, washed with water and then with a saturated solution of sodium chloride, dried over magnesium sulphate and then filtered. After evaporation of the solvent and silica gel chromatography (eluent gradient: pure cyclohexane, then cyclohexane/ethyl acetate (90/10)), the desired product is obtained as a pale yellow oil (1.05 g).

20 Yield : 64 %

Physical characteristics:

* NMR ^1H : (CDCl₃)

3.64 ppm (s, 2H); 7.01 ppm (d, 1H, $J=5.8$ Hz); 7.31 ppm (d, 1H, $J=5.8$ Hz)

* NMR ^{13}C ; (CDCl_3)

25 18.62 ppm (E); 112.28 ppm (E); 117.12 ppm (E); 127.50 ppm (O); 128.03 ppm (O); 129.80 ppm (E).

* MS: (EJ: 70eV)

203/201 ($M^{+\bullet}$; 45); 122 ($M\text{-Br}$; 100); 98 (60).

30 C/ Preparation of 2-[3'-(2'-bromo)-thiophenyl]-2-methyl-propionitrile:

To a suspension of NaH (0.140 g; 4 mmoles) in anhydrous DMF (1 ml), kept at -10°C under an inert atmosphere is added slowly (~5-10 minutes), a solution containing the preceding derivative (0.202 g; 1 mmole) and methyl iodide (0.570 g, 4 mmoles) in anhydrous DMF (1.5 ml). The reaction mixture is stirred at 0°C for one hour, then at ambient temperature for about 2 hours. The residue is taken up into 10 ml of water and extracted with 2 x 10 ml of ethyl

acetate. The organic phases are combined, washed with 3 x 10 ml of water and then with a saturated solution of sodium chloride, dried over magnesium sulphate and then filtered. The desired product is obtained, after evaporation of the solvent, as a yellow brown oil (0.240g). It will be used as such in the next step.

5 Yield : ~ quantitative

Physical characteristics :

* NMR ^1H : (CDCl_3)

1.80 ppm (s, 6H); 6.94 ppm (d, 1H, $J=5.9$ Hz); 7.24 ppm (d, 1H, $J=5.9$ Hz).

10 * NMR ^{13}C : (CDCl_3)

27.35 ppm; 33.68 ppm; 109.74 ppm; 122.97 ppm; 126.39 ppm; 126.54 ppm; 138.32 ppm.

* MS: (EI; 70eV)

231/229 (M^{+*} ; 30); 216/214 (M-15; 70); 153/151 (M-Br; 45); 125/123 (42); 97 (100).

D/ Preparation of 2- [3'-(2'-bromo)-thienyl]-2-methyl-propylamine :

The derivative prepared in Example 1/C, (0.230 g ; 1 mmole) is dissolved in anhydrous THF (1 ml), under nitrogen. A solution of aluminium hydride AlH_3 in anhydrous THF (1.5 M; 1.0 ml; 1.5 mmoles) is added slowly to the reaction mixture at ambient temperature. The mixture is refluxed for 2 hours. Brought back to ambient temperature, the medium is first of all hydrolysed with 1 to 2 ml of water and then with 2 ml of a 2N solution of HCl. After the evaporation of the solvents, the residue is taken up into 5 ml of a 2N solution of HCl and extracted with 3 x 10 ml of TBME. The aqueous phase is made alkaline (pH = 12), and then extracted with 3 x 6 ml of ethyl acetate. The organic phases are combined, washed with 2 x 10 ml of water and then with a saturated solution of sodium chloride, dried over magnesium sulphate and then filtered. The desired product is obtained after evaporation of the solvent as a yellow oil (0.125 g).

30 Yield : 53 %

Physical characteristics :

* NMR ^1H : (CDCl_3)

1.12 ppm (bs, -NH2); 1.38 ppm (s, 6H); 2.98 ppm (s, 2H); 6.84 ppm (d, 1H, $J=5.8$ Hz); 7.16 ppm (d, 1H, $J=5.8$ Hz).

35 * NMR ^{13}C : (CDCl_3)

26.38 ppm; 40.15 ppm; 52.19 ppm; 107.04 ppm; 125.61 ppm; 129.36 ppm; 145.17 ppm.

* MS: (Cl; isobutane)

236/234 (MH⁺; 100); 220/218 (M-NH₂; 5); 154 (M-Br; 47); 125/123 (12).

5

E/ Preparation of 4,4-dimethyl-thieno-[3,2-e]-isoselenazine :

10 To a solution of potassium selenocyanate (1.0 g ; 6.9 mmoles) in anhydrous DMF (7 ml), is added the derivative prepared as in Example 1/D (0.702 g ; 3 mmoles). To this solution cooled to 5°C and under an inert atmosphere are then added copper (I) iodide (0.570 g ; 3 mmoles), then triethylamine (0.900 g ; 9 mmoles). The reaction mixture rapidly becomes deeply coloured and is then 15 kept stirred for 17 hours at ambient temperature and under nitrogen. 70 ml of an aqueous sodium cyanide solution (0.560 g ; 11.4 mmoles) are added so as to complex the copper iodide out and to thus facilitate the separation of the two phases. After decanting, the aqueous phase is extracted with 70 ml of ethyl acetate. The organic phase is washed with 5 x 50 ml of water and then with 2 x 50 20 ml of a saturated solution of sodium chloride, dried over magnesium sulphate, and then filtered. The desired product is obtained after evaporation of the solvent and silica gel column chromatography (eluent: cyclohexane/ethyl acetate (95/5)) as bright yellow crystals (0.390 g).

Yield : 56 %

25 Physical characteristics :

* m.p. °C: 51.6-51.8 °C (hexane/ethyl acetate : 75/1)

* NMR ¹H: (CDCl₃)

1.21 ppm (s, 6H); 3.18 ppm (s, 2H); 3.29 ppm (bs, -NH); 7.05 ppm (d, 1H, J=5.2 Hz); 7.27 ppm (d, 1H, J=5.2 Hz).

* NMR ¹³C: (CDCl₃)

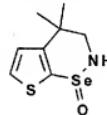
28.63 ppm (O); 33.69 ppm (E); 61.50 ppm (E); 119.78 ppm (E); 123.93 ppm (O); 127.18 ppm (O); 141.84 ppm (E).

* NMR ⁷⁷Se: (CDCl₃)

737.0 ppm.

35 * MS: (El; 70 eV)

233 (M⁺; 82); 204 (79); 189 (100); 124 (22); 97 (17).

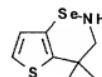
EXAMPLE 2 : Preparation of 4,4-dimethyl-thieno-[3,2-e]-isoselenazine-1-oxide:**BXT 51100**

5 The 4,4-dimethyl-thieno-[3,2-e]-isoselenazine derivative, BXT 51097, (0.115 g; 0.5 mmoles) (see Example 1) is dissolved at ambient temperature in methanol (3 ml) with stirring. After adding 5% hydrogen peroxide (0.3 ml; 0.52 mmoles), the stirring is kept up for 3 hours. The solvent is evaporated under reduced pressure. The residue is taken up into 15 ml of dichloromethane and 10 washed with 3 x 5 ml of a saturated solution of sodium chloride, dried over magnesium sulfate, and then filtered. The desired product is obtained after evaporation of the solvent as white crystals (0.065 g).

Yield : 52 %

Physical characteristics :

15 * m.p. °C: 89-90 °C
 * NMR ¹H: (CDCl₃)
 1.27 ppm (s, 3H); 1.33 ppm (s, 3H); 2.80 ppm (d, 1H, J=13 Hz); 3.86 ppm (d, 1H, J=13 Hz); 7.13 ppm (d, 1H, J=5.4 Hz); 7.54 ppm (d, 1H, J=5.4 Hz).
 * NMR ¹³C: (CH₃SeCH₃)
 20 12.82 ppm (O); 27.12 ppm (O); 35.20 ppm (E); 46.89 ppm (E); 125.96 ppm (O); 129.90 ppm (O); 131.24 ppm (E); 149.44 ppm (E).
 * NMR ⁷⁷Se: (CH₃SeCH₃)
 996.4 ppm.
 * MS: (FAB+; para-nitrobenzyl alcohol)
 25 250 (MH⁺; 40); 123 (22); 95 (50).

EXAMPLE 3: Preparation of 4,4-dimethyl-thieno-[2,3-e]-isoselenazine:**BXT 51103**

A/ Preparation of 3-bromo-2-hydroxymethyl-thiophene:

2-(3-Bromothienyl)carboxylic acid (7 g; 34 mmoles) is dissolved in 25 ml of anhydrous THF. A solution of aluminium hydride AlH_3 in anhydrous THF (2 M; 42 ml; 84 mmoles) is added slowly at a temperature of 0°C. At the end of 5 the addition, the reaction mixture is refluxed for 3 hours. After cooling to 0°C, water (200 ml) and hydrochloric acid (1N, 150 ml) are added. The mixture is decanted and the aqueous phase extracted with 3 x 150 ml of tert-butyl methyl ether. The organic phases are combined and then washed with 150 ml of a saturated solution of sodium chloride, dried over magnesium sulphate and filtered. 10 The solvent is evaporated under reduced pressure. The desired product is obtained as a brown oil and is used as such in the next step.

Yield: 95%

Physical characteristics:

* NMR ^1H : (CD_3OD)

15 4.69 ppm (s; 2H); 6.95 ppm (d; 1H, $J = 5.2$ Hz); 7.39 ppm (d; 1H, $J = 5.2$ Hz).

* NMR ^{13}C : (DMSO-d_6)

57.80 ppm; 106.28 ppm; 126.11 ppm; 129.80 ppm; 141.15 ppm.

* MS: (EI, 70 eV)

194/192 (M^{+} *; 80%); 177/175 (30%); 113 (50%); 98 (60%); 85 (100%).

20

B/ Preparation of 3-bromo-2-chloromethyl-thiophene:

The preceding derivative (6.2 g; 32 mmoles) is dissolved in anhydrous dichloromethane (180 ml). Thionyl chloride (5.7 g; 3.5 ml; 48 mmoles) is added slowly, the stirring is then kept up for 18 hours at ambient temperature. The 25 reaction mixture is poured into 200 ml of water and then decanted. The aqueous phase is extracted with 2 x 100 ml of tert-butyl methyl ether. The organic phases are combined, washed with a saturated solution of sodium bicarbonate (200 ml), dried over magnesium sulphate and filtered. The solvent is evaporated under reduced pressure. The desired product is obtained as a brown oil and is used as such for the next step.

Yield: 90 %

Physical characteristics:

* NMR ^1H : (CDCl_3)

4.75 ppm (s; 2H); 6.95 ppm (d; 1H, $J = 5.2$ Hz); 7.30 ppm (d; 1H, $J = 5.2$ Hz).

35

* NMR ^{13}C : (CDCl_3)

39.5 ppm; 112.5 ppm; 127.5 ppm; 131.0 ppm; 135.0 ppm.

* MS: (EI, 70 eV)
212 (M⁺*; 35%); 177(100%); 96 (20%).

C/ Preparation of 2-(3-bromo)-thienyl-acetonitrile:

5 The preceding derivative (6 g; 28 mmoles) is dissolved in DMSO (100 ml). Sodium cyanide (2 g; 41 mmoles) is added and stirring is kept up for 2 hours. The reaction mixture is poured into 200 ml of water. The mixture is extracted with 3 x 100 ml of tert-butyl methyl ether and then 2 x 100 ml of dichloromethane. The organic phases are combined, washed with 3 x 200 ml of water, dried over magnesium sulphate and filtered. The solvent is evaporated under reduced pressure. The residue is taken up into 400 ml of cyclohexane and washed with 4 x 100 ml of water. The organic phase is dried over magnesium sulphate and filtered. The solvent is evaporated off under reduced pressure. The desired product is obtained as a colourless oil after distillation of the residue in a 10 15 Kügelrohr distillation apparatus (T = 200°C, p = 0.1 mbar).

Yield: 60%

Physical characteristics:

* NMR ¹H: (CDCl₃)
3.84 ppm (s; 2H); 7.00 ppm (d; 1H, J = 5.2 Hz); 7.31 ppm (d; 1H, J = 5.2 Hz).
20 * NMR ¹³C: (CDCl₃)
18.39 ppm; 112.29 ppm; 116.30 ppm; 126.37 ppm; 126.65 ppm; 130.92 ppm.
* MS: (EI, 70 eV)
203/201 (M⁺*; 29%); 122 (100%); 98 (40%).

25 D/ Preparation of 2-[2'-(3'-bromo-thienyl)]-2-methyl-propionitrile:

This compound is obtained according to a procedure very similar to that of Example 1/C from the preceding derivative in the form of a yellow oil.

Yield: 90%

Physical characteristics:

30 * NMR ¹H: (acetone-d₆)
1.77 ppm (s; 6H); 7.02 ppm (d; 1H, J = 5.2 Hz); 7.44 ppm (d; 1H, J = 5.2 Hz).
* NMR ¹³C: (CDCl₃)
20.11 ppm; 33.90 ppm; 109.68 ppm; 122.15 ppm; 124.39 ppm; 133.21 ppm;
137.27 ppm.
35 * MS: (EI, 70 eV)
231/229 (M⁺*; 50%); 216/214 (100%); 189/187 (45%).

E/ Preparation of 2-[2'-(3'-bromo-thienyl)]-2-methyl-propylamine:

This compound is obtained according to a method very similar to that of Example 1/D from the preceding derivative as a pale yellow oil.

5 Yield: 80%

Physical characteristics:

* NMR ^1H : (CDCl₃)

1.10 ppm (bs; 2H); 1.44 ppm (s; 6H); 3.06 ppm (s; 2H); 6.93 ppm (d; 1H, J = 5.2 Hz); 7.07 ppm (d; 1H, J = 5.2 Hz).

10 * NMR ^{13}C : (CDCl₃)

26.45 ppm; 40.76 ppm; 51.78 ppm; 105.89 ppm; 123.01 ppm; 133.26 ppm; 145.00 ppm.

* MS: (CI, isobutane)

236/234 (MH⁺; 40%); 154 (25%); 93 (100%).

15

F/ Preparation of 4,4-dimethyl-thieno-[2,3-e]-isoselenazine: BXT 51103

This compound is obtained according to a method very similar to that of Example 1/E from the preceding derivative as a yellow oil crystallising at -20°C.

20 Yield: 54%

Physical characteristics:

* m.p. °C: 31°C

* NMR ^1H : (CDCl₃)

1.33 ppm (s; 6H); 3.20 ppm (s; 2H); 3.30 ppm (bs; 1H); 6.68 ppm (d; 1H, J = 5.3 Hz); 7.24 ppm (d; 1H, J = 5.3 Hz).

* NMR ^{13}C : (CDCl₃)

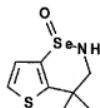
30.02 ppm; 34.16 ppm; 61.35 ppm; 119.64 ppm; 122.89 ppm; 124.57 ppm; 139.89 ppm.

* NMR ^{77}Se : (CH₃SeCH₃)

30 727.0 ppm.

* MS: (EI, 70 eV)

233 (M⁺•; 86%); 203 (10%); 189 (40%); 153 (20%), 124 (40%); 77 (35).

EXAMPLE 4: Preparation of 4,4-dimethyl-thieno-[2,3-e]-isoselenazine-1-oxide:**BXT 51105**

5

This compound is obtained according to a method very similar to that of Example 2 from the preceding derivative (BXT 51103 : see Example 3) as a colourless oil.

Yield: quantitative

10 Physical characteristics:

* NMR ^1H : (CDCl_3)

1.33 ppm (s; 3H); 1.38 ppm (s; 3H); 2.82 ppm (d; 1H, $J = 13$ Hz); 3.78 ppm (d; 1H, $J = 13$ Hz); 4.90 ppm (bs; 1H); 7.09 ppm (d; 1H, $J = 5.4$ Hz); 7.26 ppm (d; 1H, $J = 5.4$ Hz).

15

* NMR ^{13}C : (CDCl_3)

29.29 ppm; 29.45 ppm; 36.28 ppm; 48.03 ppm; 124.68 ppm; 126.03 ppm; 131.52 ppm; 153.94 ppm.

* NMR ^{77}Se : (CH_3SeCH_3)

988.20 ppm.

20

* MS: (Cl; isobutane)

250 (MH^+ ; 40%); 234 (100%); 93 (40%).

II/ Activities:

25

EXAMPLE 5 : MEASUREMENT OF THE GLUTATHIONE PEROXIDASE ACTIVITY OF COMPOUNDS OF GENERAL FORMULA I

The glutathione peroxidase activity is determined by using a 50 mM HEPES buffer, pH=7.3 (at 37°C), containing 0.2 mM DTPA, 0.144 mM NADPH, 2.2 mM reduced glutathione (GSH) and 1.1 U/ml glutathione disulphide reductase. This buffer further contains 110 U/ml catalase when hydrogen peroxide is not used as substrate.

To 1.5 ml of the buffer described above, are added 100 μl of an ethanolic stock solution of the compound tested or 100 μl of absolute ethanol (blank). Each compound is tested at a final concentration of 20 μM . The reaction

medium is equilibrated for 2 minutes at 37°C. Then 50 μ l of 6.6 mM tert-butyl hydroperoxide (t-BuOOH) in ultrapure water, or 3.3 mM hydrogen peroxide (H_2O_2) in ultrapure water are added. The glutathione peroxidase activity is determined at 37°C by measuring the decrease of absorbance at 340 nm for 5 minutes. Said activity or initial enzymatic rate is proportional to the slope of the variation of absorbance with time.

10 The catalytic activity of oxygen reduction of the compounds tested corresponds to the rate of consumption of NADPH in the absence of hydroperoxide. When this rate is significantly greater than that of the control, the corresponding glutathione oxidase activity can be checked by the direct measurement of the kinetics of consumption of the dissolved oxygen with the aid of a Clark electrode.

15 The results of glutathione peroxidase activity measurements are shown in Table 1 below. They are expressed in nmoles of hydroperoxide reduced per minute.

TABLE 1

Glutathione peroxidase activity (in nmoles of hydroperoxide reduced /min)		
	t-BuOOH	H_2O_2
BXT-51097	11.4	39.8
BXT-51100	10.1	37.1
BXT-51103	15.5	34.9
BXT-51105	13.9	35.7

20 As shown in Table 1 the compounds of general structure I described in the present invention catalyze, in the presence of glutathione GSH, the reduction of hydrogen peroxide or that of an organic hydroperoxide.

25 **EXAMPLE 6: INHIBITION OF THE TNF- α -INDUCED RELEASE OF INTERLEUKIN 8 BY ENDOTHELIAL CELLS**

It is well known to the person skilled in the art that the release of interleukin 8 (IL8) causes a massive accumulation of activated neutrophils and

thus participates in the inflammatory process and/or tissue degradation process (see Baggolini M. *et al.*; FEBS Letters, 307, 1, 97-101, 1992).

Human endothelial cells are grown at 37°C in multi-well plates under a water-saturated atmosphere constituted of a gaseous mixture of 95% air and 5% CO₂. Their culture medium is constituted by a medium M 199 pH = 7.4 containing 20% foetal calf serum, 2 mM L-glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin and 1% by volume of a medium supplement containing heparin and a growth factor for these cells.

When the cells are close to confluence, they are incubated for one hour in the presence or in the absence of one of the following compounds: BXT-51097 or BXT-51103. Each one of these compounds is incorporated at 10 µM in the culture medium containing 2% foetal calf serum, everything else being equal. After removal of the culture medium, the cells are incubated in the presence or in the absence (control) of TNF-α, at 1 ng/ml, in the same culture medium as before. In the case of cells pre-treated with a compound of the invention, the medium further contains the same compound at 10 µM. After three hours and thirty minutes of incubation, the interleukin 8 (IL-8) released into the culture medium is determined by ELISA.

The results obtained are given in the annexed Figure 1. These results show that the incubation of the endothelial cells in the presence of TNF-α leads to an increase in the production of IL-8 in the culture medium, and that the treatment of the cells by the compounds BXT-51097 and BXT-51103 inhibits this effect by at least 85%.

These results demonstrate that such compounds can act as TNF-α antagonists in terms of interleukin 8 release by endothelial cells.

EXAMPLE 7 : INHIBITION OF TNF-α-INDUCED P- AND E-SELECTIN EXPRESSIONS BY ENDOTHELIAL CELLS

It has been shown that the initial phase of inflammation is mediated by adhesion molecules of the E- and/or P-selectin type (see ALBELDA S.M. *et al.*, FASEB Journal, 8, 504-512, 1994).

Human endothelial cells are grown under the same conditions as those described in Example 6.

When the cells are close to confluence, they are incubated for one hour in the presence or in the absence of one of the following compounds: BXT-51097 or BXT-51103. Each one of these compounds is incorporated at 10 µM in the

culture medium containing 2% foetal calf serum, everything else being equal. After the removal of the culture medium, the cells are incubated in the presence or in the absence (control) of TNF- α , at 1 ng/ml, in the same culture medium as before. In the case of cells pre-treated with a compound, the medium further 5 contains the same compound at 10 μ M. After three hours and thirty minutes of incubation, the cells are washed with PBS buffer and they are fixed with 2% formaldehyde in the same buffer. The P- and E-selectin expressions on the cells are then measured by an ELISA determination by successively incubating the cells in the presence of a mouse monoclonal antibody, anti-P-selectin and anti-E- 10 selectin respectively, and a rabbit anti-mouse anti-antibody labelled with alkaline phosphatase. The quantification is carried out upon the addition of para-nitrophenyl phosphate whose hydrolysis is followed at 405 nm.

The results obtained corresponding to the measurements of P- and E-selectin expressions are shown in the annexed Figures 2 and 3 respectively. These 15 results show that the incubation of endothelial cells in the presence of TNF- α induces the expression of P- and E-selectin, which is in both cases totally inhibited when the cells are treated with the compounds BXT-51097 and BXT-51103.

The results show that such compounds are capable of inhibiting the TNF- α -induced expression of cell adhesion molecules such as P- and E-selectin.

20 The whole of these results show that the compounds of the invention having formula I:

1/ catalyse the reduction of hydroperoxides in the presence of glutathione;

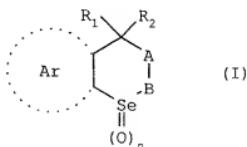
2/ antagonize the action of TNF- α ;

25 3/ inhibit the expression of cell adhesion molecules.

TNF- α , as well as the expression of adhesion molecules such as P- and E-selectins, having been implicated in pathologies which involved an over-production of hydroperoxides, the molecules of the present invention of the general formula I therefore constitute valuable active principles and, after 30 formulation, powerful drugs enabling the treatment of the corresponding pathologies.

CLAIMS

1. Cyclic organoselenium compounds of the general formula (I) :



5

in which :

R₁ = hydrogen ; lower alkyl ; optionally substituted aryl ; optionally substituted lower aralkyl ;

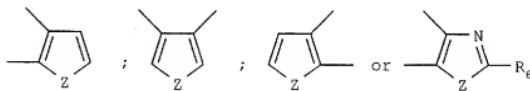
10 R₂ = hydrogen ; lower alkyl ; optionally substituted aryl ; optionally substituted lower aralkyl ;

A = CO ; (CR₃R₄)_m ;

B = NR₅ ; O ; S ;

Ar = optionally substituted phenyl or an optionally substituted radical of formula :

15



in which :

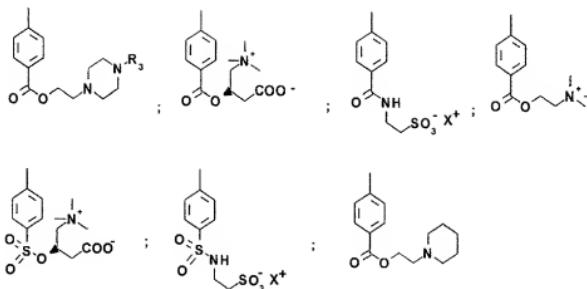
Z = O ; S ; NR₅ ;

20 R₃ = hydrogen ; lower alkyl ; optionally substituted aryl ; optionally substituted lower aralkyl ;

R₄ = hydrogen ; lower alkyl ; optionally substituted aryl ; optionally substituted lower aralkyl ;

R₅ = hydrogen ; lower alkyl ; optionally substituted aryl ; optionally substituted lower aralkyl ; optionally substituted heteroaryl ; optionally substituted lower heteroaralkyl ; CO(lower alkyl) ; CO(aryl) ; SO₂(lower alkyl) ; SO₂(aryl) ;

25 R₆ = hydrogen ; lower alkyl ; optionally substituted aryl ; optionally substituted lower aralkyl ; optionally substituted heteroaryl ; optionally substituted heteroaralkyl ; trifluoromethyl ;



m = 0 or 1 ;

n = 0 or 1 ;

5 X⁺ represents the cation of a pharmaceutically acceptable base ;
and their pharmaceutically acceptable salts of acids or bases ;
with the proviso that :
when B = NR₅ with R₅ = hydrogen, lower alkyl, optionally substituted lower aralkyl, CO(lower alkyl), and A = CO or (-CH₂)_m then Ar is different from an
10 optionally substituted phenyl.

2. A cyclic organoselenium compound of the general formula (I),
according to claim 1, selected from the group consisting in :

- the 4,4-dimethyl-thieno-[3,2-e]-isoselenazine ,
- the 4,4-dimethyl-thieno-[3,2-e]-isoselenazine-1-oxide,
- the 4,4-dimethyl-thieno-[2,3-e]-isoselenazine ,
- the 4,4-dimethyl-thieno-[2,3-e]-isoselenazine-1-oxide.

15 3. Use of cyclic organoselenium compounds, of the general formula (I), according to claim 1 or 2, as antioxidants ;
intended in particular to be added to preserving media of grafts for transplantation
20 of organs of human or animal origin such as the heart, the liver, the kidney and the
lungs ;
and

in particular for the manufacture of pharmaceutical compositions with antioxidant
activity, intended especially :

25 * for treatments of any physiopathological condition in which an over-production of cytotoxic hydroperoxides contributes to the functional impairments of cells or tissues ; and more particularly including :

- * the treatment of inflammatory and/or ischemic cardio- and cerebro-vascular pathologies, such as the preventive and/or curative treatment of arterial restenoses following an angioplasty, the preventive and/or curative treatment of arterial stenoses following artery allografts, the treatment of intermittent 5 claudication in patients affected with obstructive ischemia of the lower members, the treatment of cerebro-vascular accidents of ischemic origin ;
 - * the treatment of inflammatory and/or ischemic digestive pathologies, such as the treatment of acute inflammations of the bowel (Crohn's disease, hemorrhagic rectocolitis) ;
- 10 * the treatment of inflammatory and/or ischemic respiratory pathologies, such as the treatment of adult respiratory distress syndrome (ARDS) and infant respiratory distress syndrome (IRDS) ;
 - * the treatment of inflammatory and/or ischemic ophthalmic pathologies, such as the treatment of glaucoma ;
- 15 * the treatment of cataracts ;
 - * the treatment of acute ophthalmic allergies ;
 - * the treatment of impairments of the retina which are associated with a macular degeneration ;
 - * the treatment of viral infections causing an immuno-deficiency, such 20 as the treatment of AIDS ; and
 - * the treatment of post-radiotherapy fibroses.
- 4. Use of cyclic selenium compounds, of the general formula (I), according to claim 1 or 2, for the manufacture of pharmaceutical compositions intended for the treatment :
 - * of inflammatory diseases of the bowel such as Crohn's disease or hemorrhagic rectocolitis ;
 - * of adult respiratory distress syndrome and infant respiratory distress syndrome ;
 - * of cataracts ;
 - * of AIDS ;
 - * of post-radiotherapy fibroses.
 - 25 5. A pharmaceutical composition, notably having an antioxidant activity, characterized in that it comprises, as active ingredient, at least one cyclic organoselenium compound, of the general formula (I), according to claim 1 or 2, or one of its pharmaceutically acceptable salts of an acid or a base, optionally 30 incorporated in a pharmaceutically acceptable excipient, carrier or vehicle.

6. The pharmaceutical composition according to claim 5, characterized in that said cyclic organoselenium compound of the general formula (I) above, optionally as salt, is present in an amount between 0.1 and 5 % by weight, advantageously between 0.1 and 1 % by weight, based on the total weight of the composition.

7. The pharmaceutical composition according to one of claims 5 or 6, characterized in that it is in the form of a unit dose comprising from 1 to 500 mg of at least one cyclic organoselenium compound of the general formula (I).

8. The pharmaceutical composition according to any one of claims 5 to 7, characterized in that it is a pharmaceutical composition for the treatment of any physiopathological condition in which an over-production of cytotoxic hydroperoxides contributes to the functional impairments of cells or tissues ; this over-production of hydroperoxide can be due :

- to the activation of the intra-cellular metabolic pathways such as those of the flavine or cytochrome P-450 oxygenases, those of the lipoxygenases, and those of the monoamine oxidases ;

- to the activation of enzymes contained in endothelial cells (xanthine oxidase, 15-lipoxygenase) or in blood platelets (cyclooxygenase and 12-lipoxygenase) ;

- to the activation, by cytokines such as TNF- α , of inflammatory and/or immune cells such as neutrophils, macrophages or lymphocytes ;

- to an intoxication by a free-radical generating xenobiotic ;

- to a voluntary irradiation, such as practised during a radiotherapy, or an accidental irradiation.

9. The pharmaceutical composition according to any one of claims 5 to 8, characterized in that it is a pharmaceutical composition for the treatment of any physiopathological condition in which an over-production of cytotoxic hydroperoxides contributes to the functional impairments of cells or tissues ; and more particularly :

* for the treatment of inflammatory and/or ischemic cardio- and cerebro-vascular pathologies, such as the preventive and/or curative treatment of arterial restenoses following an angioplasty, the preventive and/or curative treatment of arterial stenoses following artery allografts, the treatment of intermittent claudication in patients affected with obstructive ischemia of the lower members, the treatment of cerebro-vascular accidents of ischemic origin ;

* for the treatment of inflammatory and/or ischemic digestive pathologies, such as the treatment of acute inflammations of the bowel (Crohn's disease, hemorrhagic rectocolitis) ;

5 * for the treatment of inflammatory and/or ischemic respiratory pathologies, such as the treatment of adult respiratory distress syndrome (ARDS) and infant respiratory distress syndrome (IRDS) ;

* for the treatment of inflammatory and/or ischemic ophthalmic pathologies, such as the treatment of glaucoma ;

* for the treatment of cataracts ;

10 * for the treatment of acute ophthalmic allergies ;

* for the treatment of impairments of the retina which are associated with a macular degeneration ;

* for the treatment of viral infections causing an immuno-deficiency, such as the treatment of AIDS ; and

15 * for the treatment of post-radiotherapy fibroses.

10. A method of preparation of cyclic organoselenium compounds, of the general formula (I), according to claim 1 or 2, characterized in that it comprises the following essential steps :

20 a/ preparing or using an orthohalo(hetero)arylacetonitrile derivative, optionally mono- or gem- disubstituted in the benzylic position; then, according to the series considered :

- for the preparation of said compounds of formula (I) in which $A=(CR_3R_4)_m$ with $m=0$ (A does not exist) and $n=0$:

b1/ hydrolyzing said nitrile derivative into an amide derivative,

25 c1-1/ transforming this into an amine derivative by a transposition reaction according to conventional methods,

d1-1/ allowing said amine derivative to react with a nucleophilic selenium derivative, optionally generated *in situ*, in the presence of a copper Cu(I) salt, in a polar organic solvent, to lead to the corresponding (hetero)arylisoselenazoline derivative,

30 e1-1/ optionally, N-alkylating or N-arylation or N-acylating or N-sulphonylating, according to conventional procedures, said (hetero)arylisoselenazoline derivative ;

- for the preparation of said compounds of formula (I) in which $A=CO$ and $n=0$:

35 b1/ hydrolyzing said nitrile derivative into an amide derivative,

c1-2/ allowing said amide derivative to react with a nucleophilic selenium derivative, optionally generated *in situ*, in the presence of a copper Cu(I) salt, in a polar organic solvent, to lead to the corresponding (hetero)arylisoselenazone derivative,

5 d1-2/ optionally, N-alkylating or N-arylation or N-acylating or N-sulphonylating, said (hetero)arylisoselenazone derivative;

- for the preparation of said compounds of formula (I) in which A = CH₂ and n = 0 :

10 b2/ reducing said nitrile derivative into an amine derivative, with the aid of borane for example in an ethereal solvent such as tetrahydrofuran for example,

15 c2/ allowing said amine derivative to react with a nucleophilic selenium derivative, optionally generated *in situ*, in the presence of a copper Cu(I) salt, in a polar organic solvent, to lead to the corresponding (hetero)arylisoselenazine derivative,

d2/ optionally, N-alkylating or N-arylation or N-acylating or N-sulphonylating, according to conventional procedures, said (hetero)arylisoselenazine derivative;

- for the preparation of said compounds of formula (I) in which A = (CR₃R₄)
20 (\neq CH₂) and n = 0 :

b3/ carrying out a mono- or a bis-C-alkylation of said nitrile derivative according to conventional methods, with the aid of an organolithium derivative for example in an ethereal solvent such as tetrahydrofuran;

25 c3/ allowing the amine derivative obtained to react with a nucleophilic selenium derivative, optionally generated *in situ*, in the presence of a copper Cu(I) salt, in a polar organic solvent, to lead to the corresponding (hetero)arylisoselenazine derivative;

30 d3/ optionally, N-alkylating or N-arylation or N-acylating or N-sulphonylating, according to conventional procedures, said corresponding (hetero)arylisoselenazine derivative ;

finally, optionally, for the preparation of compounds of formula (I) in which n = 1, Se-oxidising, according to conventional procedures, the corresponding derivative of formula (I) (wherein n = 0) obtained previously according to any one of the synthetic routes above.

35 11. Antioxidant agents comprising a cyclic organoselenium compound of formula (I) of claim 1 or 2.

12. A method for storing a graft for transplantation of organs of human or animal origin by placing said graft in an adapted storage medium comprising the step of adding to said storage medium an effective amount of at least one compound of formula (I) of claim 1 or 2.

5 13. The method of claim 12 wherein said graft is selected from the group consisting of the heart, the liver, the kidney and the lungs.

14. A method of treatment of a patient suffering from functional impairments of cells or tissues related to an over-production of cytotoxic hydroperoxides, comprising administering to said patient an effective amount of at 10 least one compound of formula (I) of claim 1 or 2.

15. The method of claim 14 wherein said patient is suffering of a pathology selected from the group consisting of :

* inflammatory and/or ischemic cardio- and cerebro-vascular pathologies ;

15 * inflammatory and/or ischemic digestive pathologies ;

* inflammatory and/or ischemic respiratory pathologies ;

* inflammatory and/or ischemic ophthalmic pathologies ;

* cataracts ;

* acute ophthalmic allergies ;

20 20 * impairments of the retina which are associated with a macular degeneration ;

* viral infections causing an immuno-deficiency ; and

* post-radiotherapy fibroses.

25 16. The method of claim 14 wherein said patient is suffering of a pathology selected from the group consisting of :

* the inflammatory diseases of the bowel ;

* the adult respiratory distress syndrome and infant respiratory distress syndrome ;

* the cataracts ;

30 * the AIDS, and

* the post-radiotherapy fibroses.

17. The method of claim 16, wherein said pathology is Crohn's disease or hemorrhagic rectocolitis.

35 18. The method of claim 14 wherein the over-production of cytotoxic hydroperoxides is due :

- to the activation of the intra-cellular metabolic pathways such as those of the flavine or cytochrome P-450 oxygenases, those of the lipoxygenases, and those of the monoamine oxidases ;
- 5 - to the activation of enzymes contained in endothelial cells (xanthine oxidase, 15-lipoxygenase) or in blood platelets (cyclooxygenase and 12-lipoxygenase) ;
- to the activation, by cytokines such as TNF- α , of inflammatory and/or immune cells such as neutrophils, macrophages or lymphocytes ;
- 10 - to an intoxication by a free-radical generating xenobiotic ;
- to a voluntary irradiation, such as practised during a radiotherapy, or an accidental irradiation.

Figure 1

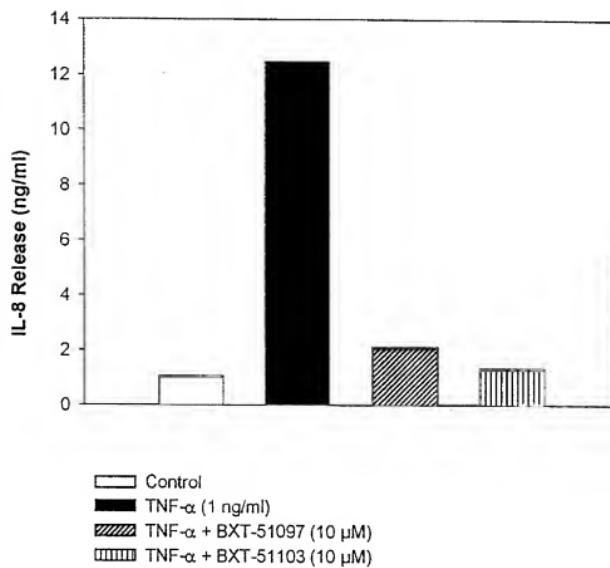


Figure 2

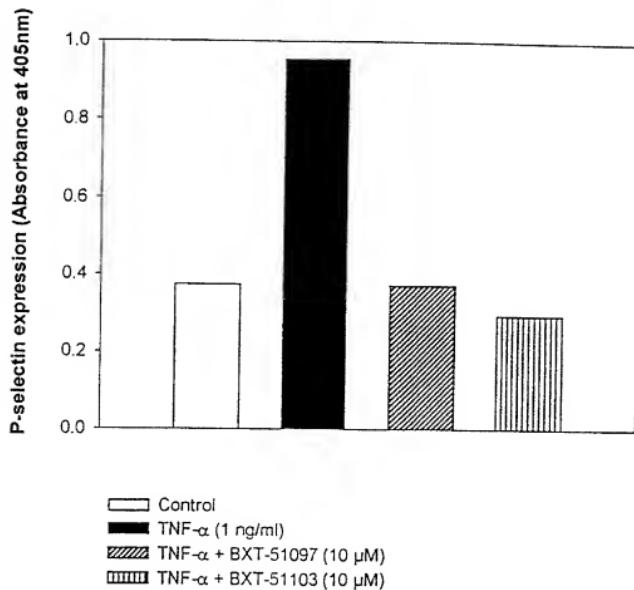
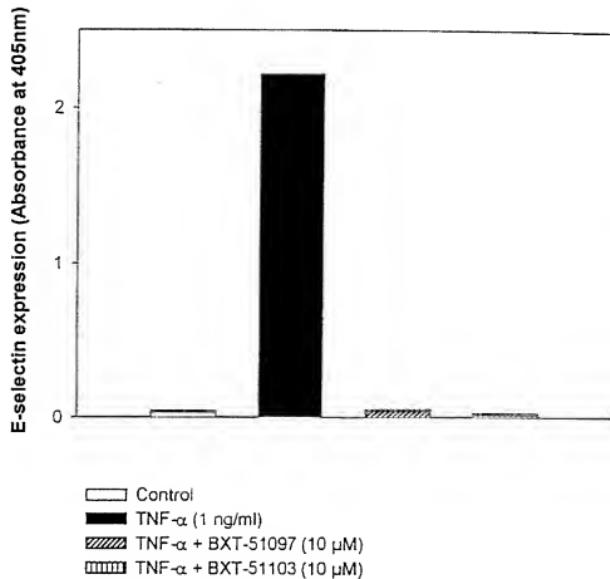


Figure 3



INTERNATIONAL SEARCH REPORT

In.	JTC	Application No
PCT/EP 97/07295		

A. CLASSIFICATION OF SUBJECT MATTER
 IPC 6 C07D517/04 C07D293/10 C07D293/12 A61K31/395 A61K31/38
 A61K31/33 C07D329/00 C07D343/00 //((C07D517/04,333:00.
 293:00))

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
 IPC 6 C07D A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 95 27706 A (BIOXYTECH) 19 October 1995 cited in the application see claims ---	1-18
Y	LAMBERT C ET AL: "Stabilisation of condensed selenium heterocycles by a nitro group in the ortho-position to the chalcogen" TETRAHEDRON, vol. 47, no. 43, 1991, pages 9053-9060, XP002027354 see the whole document ---	1-18 -/-

Further documents are listed in the continuation of box C. Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubt on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cannot be understood as involving the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
 Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

Z document member of the same patent family

2

Date of the actual completion of the international search Date of mailing of the international search report

20 April 1998

24.04.98

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Henry, J

INTERNATIONAL SEARCH REPORT

In. No. Application No
PCT/EP 97/07295

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
Y	STEPHEN R. WILSON ET AL: "Development of synthetic compounds with glutathione peroxidase activity" JOURNAL OF THE AMERICAN CHEMICAL SOCIETY, vol. 111, no. 15, 19 July 1989, DC US, pages 5936-5939, XP002039295 cited in the application see the whole document -----	1-18
Y	PIERRE V. JACQUEMIN ET AL: "Synthesis of 2-H-3,4-dihydro-1,2-benzoselenazin-3-one and derivatives:a new heterocyclic ring system" TETRAHEDRON LETTERS, vol. 33, no. 27, 1992, OXFORD GB, pages 3863-3866, XP002039296 cited in the application see the whole document -----	1-18
Y	DE 30 27 074 A (NATTERMANN A & CIE) 18 February 1982 see the whole document -----	1-18

INTERNATIONAL SEARCH REPORT

Int'l. application No.

PCT/EP 97/07295

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

- 1 Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely
Remark: Although claims 12-18 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
- 2 Claims Nos.: because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

- 1 As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos. .
4. No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos. .

Remark on Protest

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

I. ab Application No
PCT/EP 97/07295

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9527706 A	19-10-95	FR 2718441 A AU 2311195 A CA 2164642 A DE 701554 T EP 0701554 A	13-10-95 30-10-95 19-10-95 10-10-96 20-03-96
DE 3027074 A	18-02-82	NONE	